In Mozambique, at least two different phytoplasmas induce Lethal Yellowing Type Syndromes in coconut palms

Lethal Yellowing Type Syndrome of coconut in Zambesia province, Mozambique (M. Dollet)

- **DOLLET Michel,** CIRAD, Montpellier, France
- **LOURENÇO Emanuel,** MADAL,Quelimane, Mozambique
- **MACOME Feliza**, Dir. Provincial Agric., Pemba, Mozambique
- **VAZ Antonia,** Depart. Sanidad Vegetal, Maputo, Mozambique
- **FABRE Sandrine,** CIRAD, Montpellier, France

michel.dollet@cirad.fr

Lethal Yellowing Type Syndromes- LYTS-

•The phytoplasmas associated with Coconut Lethal Yellowing (CLY) in the Caribbean form the group **16SrIV**. This group contains at least 5 subgroups.

•In Africa, several LYTS of coconut palms are similar to CLY. In Ghana -West Africa- the phytoplasmas associated with the local LYTS –Cape Saint Paul Wilt Disease (CSPWD)- are different from those of CLY and form the group **16SrXXII**.

•In East Africa, phytoplasmas are associated with a LYTS called “Lethal Disease Tanzania” (LDT) in Tanzania. The first LYTS in southern Tanzania was reported in 1942. LDT phytoplasmas probably form at least one new group,**16SrXXXI.** Recent studies along the coast revealed five LDT genotypes distributed into two subgroups: Northern and Southern groups. (Mpunami et al. 2010).

•The first record of a LYTS in Mozambique was described in Zambesia in1958. It became an important economic problem in the 1990’s.The phytoplasmas associated with the LYTS in Mozambique were claimed to be closely related to the CSPWD phytoplasmas (Mpunami et al.,1999). (Fig.1).

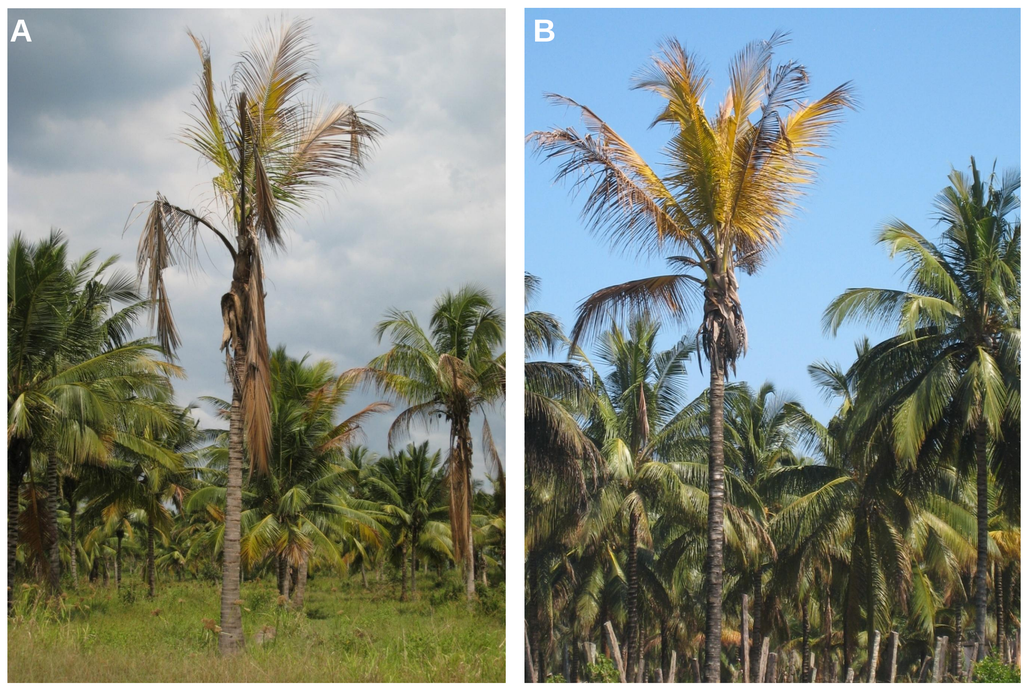

Fig.1. Final stage of LYTS in Tanzania (A) and in Mozambique (B). (M. Dollet)

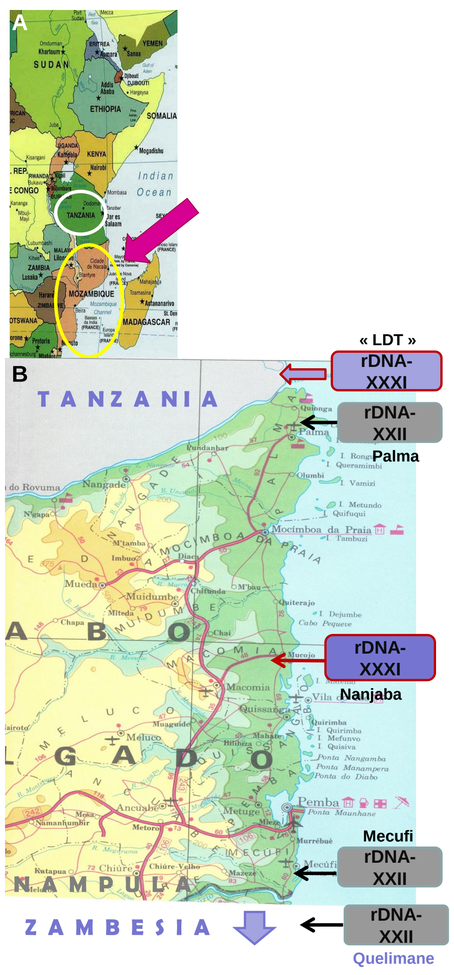

Fig. 2. Maps of East Africa (A) and North of Mozambique (Cabo Delgado province) (B)

Material and Methods

❑ Sampling

• A survey was carried out between Mecufi – South – to Palma – North, at the Tanzanian border- of Cabo Delgado province, and in the surroundings of Quelimane, in Zambesia (Fig.2).

• Coconut palms with LYTS were cut down and petioles of inflorescenses, or meristematic zone were collected and put into sterile flaks containing silicagel untill arrival at the laboratory.

❑ DNA extraction

•Total nucleic acids were obtained by the procedure derived from Daire et al. (1992) but without mercaptoetahnol and plus PVP 1%.

❑ PCR

• Samples were evaluated for phytoplasma DNA by PCR using rRNA operon primer pair P1/P7 and Rohde‘s primers suppoded to be specific of LDT (Rohde et al.1993).

❑ RFLP

• Amplicons were digested separatly with restriction endonuclases *Bam*HI, *Dra*I, *Rsa*I and *Taq*I at 37°C (65° for *Taq*I). Digest products were separated on a 2% TBE 1X agarose gel.

❑ Cloning and Sequencing

• Cloning was done in vector pGEM-T (Promega) or Topo-TA (Invitrogen).

• Sequencing was performed at Genome express.

Fig. 3. Coconut affected by a LYTS in Nanjaba, Cabo Delgado province. (M. Dollet)

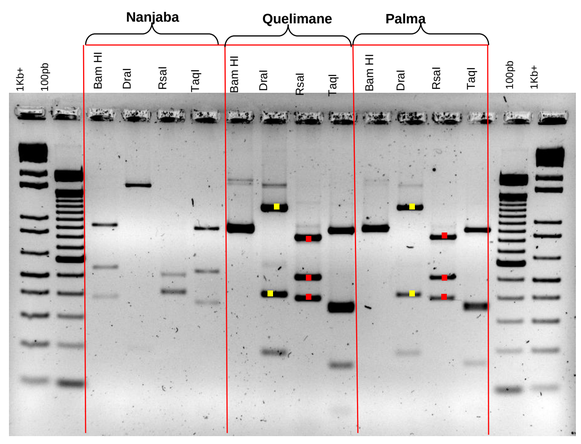

Fig. 4. RFLP on LYTS samples from Quelimane (Zambesia), Palma and Nanjaba (Cabo Delgado)

Results

• Expected 1.85 kb DNA bands were readily amplified with all the samples from Zambesia, and Cabo Delgado provinces, when using primers P1/P 7.

• Rohde’s primers, allowed amplification only with the sample from Nanjaba (Fig.3) in the middle of Cabo Delgado province (Fig.2).

• For sequences of samples from Quelimane, Mecufi and Palma, a BLAST search revealed a 99 to 100% similarity with phytoplasmas from Zambesia already sequenced in our laboratory in 2003 and 2004 and with CSPWD phytoplasmas from Ghana (**16Sr XXII**).

• For the **Nanjaba isolate**, BLAST search done on the sequences obtained with P1/P7 and Rohde’s primers, revealed **100% similarity with LDT phytoplasmas** (**16SrXXXI**).

• RFLP pattern confirmed the difference between Nanjaba isolate and the others (Fig.4).

Fig. 5 *Platacantha lutea* (Pentatomidae) on diseased coconut in Mecufi. (M. Dollet)

VECTOR(S) ?

Which vector for which phytoplasma ?

During the survey, the pentatomid bug *P. lutea* (Fig. 5) was found carrying 16Sr XXII phytoplasmas, identical to those identified in the diseased coconut on which they were feeding, in Mecufi – Cabo Delgado (Dollet et al. 2011). Search for the vector of 16SrXXII phytoplasmas in Ghana and the vector of 16Sr XXXI in Tanzania stays fruitless. As the entomofauna of these different regions (Ghana, Zambesia, Northern Tanzania) varies, we can imagine there could be different vectors.

Conclusion

Very often the single term “Lethal Yellowing “ (name of the Caribbean wilt) is used to refer to any coconut disease for which a yellowing affects some leaves, and most of plant pathologists not familiar with coconut diseases, frequently consider there is one single phytoplasma (“LY”) associated with every LYTS. This work shows that, even in a same country, two different phytoplasmas can be associated with LYTS of coconut.

This result raises the question of the insect(s) vector(s) of these different phytoplasmas in a same area. It also shows that it does not make sense to give a country name (like “LDTanzania”) to a phytoplasma disease. Insect vectors certainly have no borders!

References

- **Daire** X., Boudon-Padieu E., Berville A., Schneider B., Caudwell A. 1992. Ann. Appl. Biol.121: 95-103.
- **Dollet** M., Macome F., Vaz A., Fabre S. 2011. Bulletin of Insectology, 64: S47-S48
- **Mpunami** A., Tymon A., Jones P., Dickinson J. 1999. Plant pathol.48: 109-114.
- **Mpunami** A., Pilet F., Fabre S., Kullaya A., Dollet M. 2010. Meeting Cost Action FA087.Sitges, Spain, February 1rst-2nd 2010.
- **Rohde** W., Kullaya A., Mpunami A., Becker D. 1993. Oléagineux, 48:319-322

REPÚBLICA DE MOÇAMBIQUE
MINISTÉRIO DA AGRICULTURA
DIRECÇÃO NACIONAL DE SERVIÇOS AGRÁRIOS
DEPARTAMENTO DE SANIDADE VEGETAL

REPÚBLICA DE MOÇAMBIQUE
MINISTÉRIO DA AGRICULTURA
DIRECÇÃO PROVINCIAL DE AGRICULTURA
DE CABO-DELGADO

In Mozambique, at least two different phytoplasmas induce lethal yellowing type syndromes in coconut palms

Michel Dollet*, Emanuel Lourenço, Felizia Macome, Antonia Vaz, Sandrine Fabre.

CIRAD, UMR 98, TA A-98/F, 34398 Montpellier Cedex 5, France

Grupo Madal S.A.R.L., Quelimane, Zambezia Province, Mozambique

Provincial Service of Agriculture, Pemba, Cabo-Delgado province, Mozambique

Direcçao Nacional Servicios Agrarias, Maputo, Mozambique

Several African lethal yellowing type syndromes (LYTS) are similar to “Coconut Lethal Yellowing” (CLY) in the Caribbean that affects coconut palms. In West Africa, the phytoplasmas associated with LYTS in Ghana – Cape Saint Paul Wilt Disease (CSPWD) - were different from those of the 16SrIV subgroups associated with CLY. In East Africa a LYTS called “Lethal Disease” occurs in Tanzania (LDT). Recent studies along the coast revealed five LDT genotypes distributed into two groups (Northern and Southern groups). The phytoplasmas associated with the LYTS in Mozambique were claimed to be closely related to the CSPWD phytoplasmas and different from those causing LDT. We sampled several coconuts with LYTS in Northern Mozambique in order to validate the observation.
Sampling was performed in Zambesia around Quelimane – from Macuze to Inhassünge. In Cabo Delgado province, samples were collected from Mecufi – 40 km South of Pemba – to Palma , 160 km North of Pemba, 20 km South of Tanzania. Samples included petioles of inflorescences or trunk borings. DNA extraction was done with CTAB. PCR were performed with P1/P7 primers or Rohde‘s primers claimed to be specific for LDT. PCR products were cloned in pGEM-T. DNA sequences were submitted to a BLAST search.
Around 90% of the extracted DNAs were amplified with P1/P7. Only the samples from Nanjaba, a village 170 km south of the Tanzania border, were amplified with Rohde's primers. BLAST search with the sequences obtained in Nanjaba revealed 100% similarity with phytoplasmas of the Southern group, genotype V, from the South of Tanzania. Other samples, closer to the border of Tanzania (in Palma), showed sequences similar to the phytoplasmas found in Zambesia, which were closer to CSPWD phytoplasmas.
These results raise the question of transmission of these two phytoplasmas present in a same region in Mozambique.

IOM 2012 19th Congress of the International Organization for Mycoplasmology

15-20 July 2012 – Toulouse - France